

What is claimed is:

1. A composition comprising a recombinant invasin protein of at least 95% purity.
2. The composition of claim 1 wherein the purified recombinant invasin protein comprises an amino acid sequence derived from an invasin protein of a bacterium chosen from the group consisting of *Shigella* spp., *Salmonella* spp., and enteroinvasive *E. coli*.
3. The composition of claim 2 wherein the purified recombinant protein is an IpaC or a SipC protein.
4. The composition of claim 2 wherein the purified recombinant invasin protein comprises an amino acid sequence chosen from the group consisting of SEQ ID NO: 1 and SEQ ID NO: 2.
5. The composition of claim 2 wherein the purified recombinant invasin protein comprises a mutant selected from the group consisting of HisTag-B-C, HisTag-A-C, HisTag -A-B, IpaC $\Delta$ 1, IpaC $\Delta$ H, IpaC $\Delta$ II and IpaC $\Delta$ III.
6. The composition of claim 1 wherein the purified recombinant invasin protein is at least 97% pure.
7. The composition of claim 1 wherein the purified recombinant invasin protein comprises an amino acid sequence of at least 15 amino acids.
8. The composition of claim 1 wherein the purified recombinant invasin protein comprises an amino acid sequence of at least 20 amino acids.
9. The composition of claim 1 wherein the purified recombinant invasin protein comprises an amino acid sequence of at least 30 amino acids.

10. The composition of claim 1 wherein the purified recombinant invasin protein comprises an amino acid sequence in which no more than 35% of the amino acid residues have been conservatively substituted.

11. The composition of claim 1 wherein the purified recombinant invasin protein comprises an amino acid sequence in which no more than 10% of the amino acid residues have been conservatively substituted.

12. The composition of claim 1 wherein the purified recombinant invasin protein has adjuvant activity.

13. A method for the production of a purified recombinant invasin protein comprising:

- a) inserting a polynucleotide encoding an invasin protein into an expression vector;
- b) transforming (the combination of a) into a host cell;
- c) growing the host cell under conditions conducive to soluble protein expression;
- d) extracting the protein from a host cell lysate, culture medium, or reconstituted organism with a solution comprising a protein denaturant;
- e) performing an affinity purification of the invasin protein wherein the method of said purification is performed in the presence of a protein denaturant;
- f) removing said protein denaturant from the protein solution obtained in (the purification process of e) until the concentration of the denaturant is at the minimum concentration necessary to maintain protein solubility; and
- g) rapidly diluting the purified protein into a volume of denaturant-free solution.

14. A method for the production of a purified recombinant invasin protein comprising:

- a) combining a polynucleotide encoding the invasin protein and a polynucleotide encoding an affinity purification moiety;
- b) transforming the combination of a), in an appropriate expression vector, into a host cell;

- 10
- c) growing the host cell under conditions conducive to soluble protein expression;
  - d) extracting the protein from a host cell lysate, culture medium, or reconstituted organism with a solution comprising a protein denaturant;
  - e) performing an affinity purification of the invasin protein appropriate for the affinity purification moiety encoded by the polynucleotide in a), wherein the method of said purification is performed in the presence of a protein denaturant;
  - f) removing said protein denaturant from the protein solution obtained in the purification process of e) until the concentration of the denaturant is at the minimum concentration necessary to maintain protein solubility; and
  - g) rapidly diluting the purified protein into a volume of denaturant-free solution.

15. The method of claim 14 wherein the affinity purification moiety is His-Tag.

500  
16. The method of claim 13 or 14 wherein the protein denaturant is selected from the group consisting of guanidine hydrochloride, detergents, and urea

17. The method of claim 13 or 14 wherein the protein denaturant is urea.

18. The method of claim 17 wherein the concentration of urea is between about 1 M and about 10 M.

19. The method of claim 17 wherein the concentration of urea is between about 5 M and about 7 M.

20. The method of claim 17 wherein the concentration of urea is about 6 M.

21. The method of claim 14 further comprising the step of removing the affinity purification moiety from the recombinant invasin protein.

Sub 23  
22. The method of claim 13 or 14 wherein the dilution of the purified protein occurs in about 1 minute or less.

23. The method of claim 22 wherein the dilution of the purified protein occurs in about 30 seconds or less.

24. The method of claim 23 wherein the dilution of the purified protein occurs in about 10 seconds or less.

- ✓  
25. A method for the production of a purified recombinant invasin protein comprising:
- a) combining a polynucleotide encoding the invasin protein and a polynucleotide encoding an affinity purification moiety;
  - b) transforming the combination of a), in an appropriate expression vector, into a host cell;
  - c) growing the host cell under conditions conducive to soluble protein expression;
  - d) extracting the protein from a host cell lysate, culture medium, or reconstituted organism with a solution comprising 6 M urea;
  - e) performing an affinity purification of the invasin protein appropriate for the affinity purification moiety encoded by the polynucleotide in a), wherein the method of said purification is performed in the presence of a protein denaturant;
  - f) removing said protein denaturant from the protein solution obtained in the purification process of e) until the concentration of the denaturant is at the minimum concentration necessary to maintain protein solubility; and
  - g) diluting the purified protein in about 10 seconds or less into a volume of denaturant-free solution.

50

Sub 26. An adjuvant composition comprising at least one purified recombinant invasin protein, wherein administration of the adjuvant composition to an animal in combination with an antigen elicits an immune response to the antigen.

27. The adjuvant composition of claim 26 wherein the purified recombinant invasin protein is of at least 95% purity.

28. The adjuvant composition of claim 26 wherein the purified recombinant invasin protein is of at least 97% purity

29. The adjuvant composition of claim 26, wherein the purified recombinant invasin protein comprises an amino acid sequence derived from a protein of a member of the *Shigella* or *Salmonella* genus, or from an enteroinvasive *E. coli*.

30. The adjuvant composition of claim 29 wherein the purified recombinant protein is an IpaC or a SipC protein.

31. The adjuvant composition of claim 29 wherein the purified recombinant invasin protein comprises an amino acid sequence chosen from the group consisting of SEQ ID NO: 1 and SEQ ID NO: 2.

32. The adjuvant composition of claim 29 wherein the purified recombinant invasin protein comprises a mutant selected from the group consisting of HisTag-B-C, HisTag-A-C, HisTag-A-B, IpaCA1, IpaCAH, IpaCAII and IpaCAIII.

33. The adjuvant composition of claim 26 wherein the purified recombinant invasin protein comprises an amino acid sequence of at least 15 amino acids.

34. The adjuvant composition of claim 26 wherein the purified recombinant invasin protein comprises an amino acid sequence of at least 20 amino acids.

51

35. The adjuvant composition of claim 26 wherein the purified recombinant invasin protein comprises an amino acid sequence of at least 30 amino acids.

36. The adjuvant composition of claim 26 wherein the immune response to the antigen is a T cell response.

37. The adjuvant composition of claim 26 wherein the immune response to the antigen is a B cell response.

38. The adjuvant composition of claim 26 wherein the immune response is characterized by the production of at least one cytokine by Th2 cells.

39. The adjuvant composition of claim 38, wherein the at least one cytokine is an interleukin (IL).

40. The adjuvant composition of claim 39, wherein the interleukin (IL) chosen from the group consisting of IL-4, IL-5, IL-6, IL-10 and IL-13.

41. The adjuvant composition of claim 26, wherein the immune response is characterized by production of at least one class of immunoglobulin chosen from the group consisting of IgG, IgE, IgM and IgA.

Sub A5 ✓  
42. An adjuvant composition comprising a purified recombinant invasin protein of at least 95% purity and having adjuvant activity, the invasin protein comprising an amino acid sequence derived from a protein of a member of the *Shigella* or *Salmonella* genus, or from an enteroinvasive *E. coli* wherein administration of the adjuvant composition in combination with an antigen to an animal results in production by Th2 cells of at least one cytokine selected from the group consisting of IL-4, IL-5, IL-6, IL-10 and IL-13.

43. An adjuvant composition comprising a purified recombinant invasin protein of at least 95% purity and having adjuvant activity, the invasin protein comprising an amino acid

sequence derived from a protein of a member of the *Shigella* or *Salmonella* genus, or from an enteroinvasive *E. coli* wherein administration of the adjuvant composition in combination with an antigen to an animal results in production of at least one class of immunoglobulin selected from the group consisting of IgG, IgE, IgM and IgA.

44. A vaccine preparation comprising,

a purified recombinant invasin protein having adjuvant activity,  
at least one antigen, and  
a pharmaceutically acceptable carrier, diluent or excipient.

45. The vaccine preparation of claim 44 wherein the antigen is an infective agent, a subunit of an infective agent, a biologically active chemical, or a toxoid.

46. The vaccine preparation of claim 45 wherein the infective agent is selected from the group consisting of a bacterium, a virus, a retrovirus, a protozoan, a parasite and a fungus.

47. The vaccine preparation of claim 44 wherein the ratio of antigen to purified recombinant invasin protein is about one part antigen to between about 0.0001 to about 10,000 parts purified invasin protein.

48. The vaccine preparation of claim 44 wherein the ratio of antigen to purified recombinant invasin protein is about one part antigen to between about 0.001 to about 1,000 parts purified invasin protein.

49. The vaccine preparation of claim 44 wherein the ratio of antigen to purified recombinant invasin protein is about one part antigen to between about 0.01 to about 100 parts purified invasin protein.

50. The vaccine preparation of claim 44 wherein the purified recombinant invasin protein has a purity of at least about 95%.

51. The vaccine preparation of claim 44 wherein the purified recombinant invasin protein has a purity of at least about 97%.

52. The vaccine preparation of claim 44, wherein the purified recombinant invasin protein comprises an amino acid sequence derived from a protein of a member of the *Shigella* or *Salmonella* genus, or from an enteroinvasive *E. coli*.

53. The vaccine preparation of claim 52 wherein the purified recombinant protein is an IpaC or a SipC protein.

54. The vaccine preparation of claim 52 wherein the purified recombinant invasin protein comprises an amino acid sequence chosen from the group consisting of SEQ ID NO: 1 and SEQ ID NO: 2.

55. The vaccine preparation of claim 52 wherein the purified recombinant invasin protein comprises a mutant selected from the group consisting of HisTag-B-C, HisTag-A-C, HisTag - A-B, IpaCΔ1, IpaCΔH, IpaCΔII and IpaCΔIII.

56. The vaccine preparation of claim 44 wherein the purified recombinant invasin protein comprises an amino acid sequence of at least 15 amino acids.

57. The vaccine preparation of claim 44 wherein the purified recombinant invasin protein comprises an amino acid sequence of at least 20 amino acids.

58. The vaccine preparation of claim 44 wherein the purified recombinant invasin protein comprises an amino acid sequence of at least 30 amino acids.

59. The vaccine preparation of claim 44 wherein administration of the vaccine preparation to an animal elicits a T cell response to the antigen.



54  
60. The vaccine preparation of claim 44 wherein administration of the vaccine preparation to an animal elicits a B cell response to the antigen.

Sub 46  
61. The vaccine preparation of claim 59, wherein the immune response is characterized by the production of at least one cytokine by Th2 cells.

62. The vaccine preparation of claim 61, wherein the at least one cytokine is an interleukin (IL).

63. The vaccine preparation of claim 62, wherein the interleukin (IL) chosen from the group consisting of IL-4, IL-5, IL-6, IL-10 and IL-13.

64. The vaccine preparation of claim 44, wherein the immune response is characterized by production of at least one class of immunoglobulin directed against the antigen administered chosen from the group consisting of IgG, IgE, IgM and IgA.

Sub 47  
65. A vaccine preparation comprising,  
a purified recombinant invasin protein of at least about 95% purity and having adjuvant activity, the invasin protein comprising an amino acid sequence derived from a protein of a member of the *Shigella* or *Salmonella* genus, or from an enteroinvasive *E. coli*,  
at least one antigen, and  
a pharmaceutically acceptable carrier, diluent or excipient.

66. The vaccine preparation of claim 65, wherein administration of the vaccine preparation to an animal results in production by Th2 cells of at least one cytokine selected from the group consisting of IL-4, IL-5, IL-6, IL-10 and IL-13.

67. The vaccine preparation of claim 65, wherein administration of the vaccine preparation to an animal results in production of at least one class or subclass of immunoglobulin directed against the antigen administered selected from the group consisting of IgG, IgE, IgM and IgA.

55

Sub 08  
68. A vaccine preparation for conferring immunity against an organism expressing invasin protein antigens comprising, a purified recombinant invasin protein having adjuvant activity derived from the invasin protein antigens expressing organism against which immunity is desired.

69. The vaccine preparation of claim 44, 65 or 68, further comprising at least one additional adjuvant or immune system stimulant.

Sub 09  
70. A method for eliciting an immune response in an animal comprising, administering to an animal an immune response eliciting amount of an adjuvant composition comprising a purified recombinant invasin protein.

71. The method of claim 70 wherein the purified recombinant invasin protein has a purity of at least about 95%.

Sub 010  
72. the method of claim 70 wherein the purified recombinant invasin protein has a purity of at least about 97%.

73. The method of claim 70, further comprising administration of an antigen.

74. The method of claim 70, further comprising administration of at least one additional adjuvant or immune stimulant.

75. The method of claim 70 wherein the immune response is a T cell response.

76. The method of claim 70 wherein the immune response is a B cell response.

77. The method of claim 70 wherein the immune response involves the production of at least one cytokine by Th2 cells.

78. The method of claim 77, wherein the at least one cytokine produced is an interleukin (IL).

79. The method of claim 78, wherein the interleukin (IL) is chosen from the group consisting of IL-4, IL-5, IL-6, IL-10 and IL-13.

80. The method of claim 73 wherein the ratio of antigen to purified recombinant invasin protein is about one part antigen to between about 0.0001 to about 10,000 parts recombinant invasin protein.

81. The method of claim 70 wherein the immune response involves the production of at least one class of immunoglobulin.

82. The method of claim 81 wherein the class of immunoglobulin is chosen from the group consisting of IgG, IgE, IgM and IgA.

83. The method of claim 70 wherein the purified recombinant invasin protein comprises an amino acid sequence derived from a protein of a member of the *Shigella* or *Salmonella* genus, or from an enteroinvasive *E. coli*.

84. The method of claim 83 wherein the purified recombinant protein is an IpaC or a SipC protein.

85. The method of claim 83 wherein the purified recombinant invasin protein comprises an amino acid sequence chosen from the group consisting of SEQ ID NO: 1 and SEQ ID NO: 2.

86. The method of claim 83 wherein the purified recombinant invasin protein comprises a mutant selected from the group consisting of HisTag-B-C, HisTag-A-C, HisTag-A-B, IpaCΔ1, IpaCΔH, IpaCΔII and IpaCΔIII.

87. The method of claim 70 wherein the purified recombinant invasin protein comprises an amino acid sequence of at least 15 amino acids.

88. The method of claim 70 wherein the purified recombinant invasin protein comprises an amino acid sequence of at least 20 amino acids.

89. The composition of claim 70 wherein the purified recombinant invasin protein comprises an amino acid sequence of at least 30 amino acids.

90. A method for stimulating the production of at least one cytokine by Th2 cells comprising, administering a cytokine production stimulating amount of a purified recombinant invasin protein of at least about 95% purity comprising an amino acid sequence derived from a protein of a member of the *Shigella* or *Salmonella* genus, or from an enteroinvasive *E. coli*, wherein the cytokine produced is chosen from the group consisting of IL-4, IL-5, IL-6, IL-10 and IL-13.

91. The method of claim 90, further comprising administration of an antigen.

92. The method of claim 91 wherein the ratio of antigen to purified recombinant invasin protein is about one part antigen to between about 0.0001 to about 10,000 parts recombinant invasin protein.

93. The method of claim 90, further comprising administration of at least one additional adjuvant or immune stimulant.

94. A method for stimulating production of at least one class of immunoglobulin comprising administration of an immunoglobulin production stimulating amount of a purified recombinant invasin protein of at least 95% purity comprising an amino acid sequence derived from a protein of a member of the *Shigella* or *Salmonella* genus, or from an enteroinvasive *E. coli*, wherein the class or subclass of immunoglobulin produced is chosen from the group consisting of IgG, IgE, IgM and IgA

95. The method of claim ~~94~~, further comprising administration of an antigen.

96. The method of claim 95 wherein the ratio of antigen to purified recombinant invasin protein is about one part antigen to between about 0.0001 to about 10,000 parts recombinant invasin protein.

97. The method of claim 94, further comprising administration of at least one additional adjuvant or immune stimulant

98. A method for the delivery of pharmacologically active substances, therapeutic substances, cytotoxic substances, or diagnostic substances into cells comprising administering a pharmacologically active substance, cytotoxic substance, or diagnostic substance and a purified recombinant invasin protein.

99. The method of claim 98 wherein the pharmacologically active substance, cytotoxic substance or diagnostic substance is coupled to a purified recombinant invasin protein.

100. A method for the delivery of pharmacologically active substances, therapeutic substances, cytotoxic substances, or diagnostic substances to cells comprising a fused protein comprising a recombinant invasin protein and a pharmacologically active substance, therapeutic substance, cytotoxic substance, or diagnostic substance.